Patent Claims

1) Process for measuring apoptosis, characterised in that

A) a population of mammalian cells is transiently transfected

ai) with a plasmid containing a DNA sequence of interest which is to be investigated as to whether it or the polypeptide expressed thereby has a pro- or anti-apoptotic activity,

aii) or with a plasmid containing a DNA sequence of interest which is to be investigated as to whether, or by means of which substances, its pro- or anti-apoptotic activity or the activity of the polypeptide expressed thereby can be modulated,

b) and with a plasmid containing a DNA coding for a fluorescent marker protein,

B) in that the cells are incubated in a suitable nutrient medium, until the DNA sequence of interest or the expressed polypeptide has exerted its potential activity on the apoptosis,

C) in that the cells are harvested and fixed so that the fluorescent protein remains in the cells, while the DNA fragments formed during apoptosis are able to diffuse out of the cells,

D) in that the proportion of apoptotic cells is determined by measuring the DNA content,

E) in that the proportion of transfected cells is determined by measuring the cells with fluorescent marker protein,

F) and that by comparing the values obtained in steps D and E the proportion of apoptotic cells in the transfected subpopulation of the cells is determined.

2. Process according to claim 1, characterised in that the transfection of the cells is carried out with polyethyleneimine and inactivated Adenovirus.

3. Process according to claim 1, characterised in that the fluorescent polypeptide defined in A b) is Green Fluorescent Protein.

4. Process according to claim 1, characterised in that the DNA content is measured with a DNA-binding stain.

5. Process according to claim 4, characterised in that the stain is propidium iodide.

6. Process according to one of claims 1 to 5, characterised in that the incubation according to step B) is carried out in the presence of a test substance.

7. Process according to one of claims 1 to 6, characterised in that the incubation according to step B) is carried out in the presence of a substance which stimulates apoptosis.

8. Process according to one of claims 1 to 7, characterised in that the primary fixing in step C is carried out with paraformaldehyde and the secondary fixing/permeabilisation of the cells is carried out with ethanol.

9. Process according to one of claims 1 to 8, characterised in that the measurements defined in steps D and E are carried out in one step using fluorescence-activated throughflow cytometry analysis.

10. Kit for performing the process according to claim 1, characterised in that it contains the following components in several separate containers:

a) one or more components required for the transfection;

b) a plasmid containing the sequence coding for the fluorescent marker protein;

c) an empty vector for inserting the DNA sequence of particular interest and for control measurements;

d) the primary fixing solution;

e) the secondary fixing/permeabilising solution;

f) washing solution(s);

g) a DNA-binding stain.

11. Kit according to claim 10, containing as component a) polyethyleneimine and psoralen/UV-inactivated Adenovirus.

12. Kit according to claim 10, containing as component b) a plasmid coding for Green Fluorescent Protein.

13. Kit according to claim 10, containing as component d) an approximately 2% paraformaldehyde solution and as component e) about 70 % ethanol.

14. Use of the process according to claim 1 for identifying substances which modulate the pro- or anti-apoptotic activity of genes or gene products.

15. Use according to claim 14 for identifying therapeutically effective substances which exhibit a synergistic activity with the inhibition or absence of the survival factor function from tumour cells, characterised in that the cells are tumour cells, in that the DNA sequence according to aii) is a dominant-negative version of a receptor for a survival factor particular to a tumour cell or a signal transmission molecule of a receptor of this kind, and in that the cells are incubated in the presence of the test substance.

16. Use according to claim 15, characterised in that the DNA sequence according to aii) is a dominant-negative version of the IGF-1 receptor.

17. Use according to claim 15, characterised in that the DNA sequence according to aii) is a dominant-negative version of an FGF receptor.

18. Use according to claim 15, characterised in that the DNA sequence according to aii) is a dominant-negative version of a PDGF receptor.

add A2